2397-Pos Board B416
The Selectivity of MscS is Determined by the Cytoplasmic Domain
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The mechanosensitive channel of small conductance (MscS) is a heptameric pressure sensitive channel expressed in the inner membrane of *E. coli*. This channel possesses three transmembrane helices and a large water-filled cytoplasmic cage which comprises more than 50% of the total protein. This cytoplasmic domain is conserved throughout the MscS channel family and in MscS has been shown to be a dynamic structure with an active role in channel gating. It has also been suggested that the cytoplasmic domain determines the weak anion selectivity exhibited by MscS. In order to address this question this study reconstituted wild type MscS and single residue mutants of the cytoplasmic vestibule in azolectin liposomes (protein:lipid 1:10000). Patch clamp recordings of these channels were then performed in the presence of asymmetric solutions of KCl (600/200 mM) and $BaCl_2$ (50/200 mM). The MscS mutants studied were R184E, R185E, E187R and E227A. Both E187R and E227A mutants show reduced selectivity characterised by a lower anion-cation permeability ratio. These residues are likely to determine selectivity by binding cations. From these data it is clear that charged residues in the cytoplasmic domain of MscS determine its selectivity. This is interesting because unlike K^+, Na^+ and Ca^{2+} channels the selectivity of MscS is not determined by residues in the pore region but residues situated in the large water-filled cytoplasmic domain.

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The Right-Side-Out Orientation of MscS in Liposomal Membranes
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The bacterial mechanosensitive channel MscS plays a crucial role in the protection of bacterial cells against hypo-osmotic shock. MscS functional characteristics have extensively been studied in both giant spheroplasts and liposomes. Despite many studies of MscS reconstituted into liposomes the channel orientation in liposomal membranes is still unknown. We examined the orientation of MscS in liposomes by patch-clamp and confocal microscopy. using its previously determined electrophysiological and pharmacological properties we were able to determine that in liposomes MscS retains the right-side-out orientation as in giant spheroplasts based on the following evidence: (i) I-V curves recorded in both spheroplast and liposome preparations exhibited strong outward rectification at both negative and positive pipette pressures. (ii) MscS activation ratio in liposome patches at positive relative to negative pipette voltages and *vice versa* showed positive correlation at both positive and negative pipette pressures similar to MscS in inside-out excised spheroplast patches. (iii) MscS exhibited a voltage-dependent hysteresis upon application of sawtooth pressure ramps in both spheroplasts and liposomes. In both spheroplasts and liposomes the hysteresis was more pronounced upon positive pipette voltages compared to negative voltages. (iv) 2.5% of 2,2,2-trifluoroethanol (TFE) caused MscS inactivation in liposome patches when added to the cytoplasmic side of MscS, whereas addition of TFE to the periplasmic side did not inactivate the channel, although it caused a shift of the channel activation towards lower pipette pressures. We obtained a similar result when applying TFE to MscS in spheroplast patches. In conclusion, our findings strongly indicate that the cytoplasmic domain of MscS in liposome membrane patches faces the bath solution as in spheroplast patches. Consequently, upon liposome reconstitution MscS channels preserve their right-side-out orientation comparable to what was previously reported for the MscL channels.
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Modulation of the G22E MscL Mutant Channel Gating by Lipid Bilayer Constituents and Gadolinium
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Bacteria respond to hypoosmotic changes through the mechanosensitive (MS) channels of Large (MscL) and Small (MscS) conductance, MscS responds first to pressure, i.e. bilayer tension changes, followed by MscL[1]. The lipid environment, lyso lipids and cholesterol have been shown to significantly influence the ratio of the opening of MscL to MscS[2,3]. Furthermore, introduction of the highly negatively charged cardiolipin to both azolectin and POPE/POPC lipid membranes causes rapid gating of MscS[4]. Here we report an expanded study using the spontaneously active G22E MscL mutant which, although spontaneously active, is still mechanosensitive. Addition of sub-millimolar amounts of the metal ion Gadolinium(III) reversibly inhibits spontaneous channel activity, but upon application of pressure, the channel exhibits mechanosensitivity similar to the wild-type MscL. Our results are consistent with the previous studies showing that Gd(III) inhibits MscL mechanosensitivity by binding to the lipid bilayer[5,6]
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2400-Pos Board B419
A Novel Approach to follow Helical Movements of an Ion Channel in Real-Time
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Mechanosensitive channel of large conductance (MscL) is one of the best-studied mechanosensitive channels in bacteria (Sukharev et al., 1994, Blount et al., 2007). It acts as a safety valve in response to hyperosmotic shock. High-resolution structure of Mycobacterium tuberculosis MscL revealed that it forms a homopentamer with two transmembrane helices per subunit. Although in nature the channel opens in response to tension, breaking the hydrophobic interactions at its pore region leads to the spontaneous opening of the channel. Here, by using this principle, we modified the hydrophobic gate of the channel with designed chemical switches and we gained external control over its activation. We followed the resulting structural changes on the protein by following the Electron Paramagnetic Resonance signal from a spin label on different positions at the pore forming helices. We developed a method in which we could control the number of switches and EPR spin labels per pentamer. By this approach, we start following the gradual activation of the channel in real time.

2401-Pos Board B420
MscL as a Triggered Nanovalve: New Modifications to Improve Design
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MscL is a small homopentameric bacterial protein that has, among other characteristics, an incredible pore size greater than 30Å and the ability to gate in response to mechanical tension in the membrane. Because of its amenability, *E.coli* MscL has been the most studied mechanosensitive channel, serving as a paradigm of how a protein can sense and transduce mechanical force. Early on in the study of the channel a critical domain for MscL gating was revealed by forward genetic experiments screening for mutations that led to a gain-of-function (slowed- or no-growth) phenotypes: mutations at residue G22, within the pore, led to severe gain-of-function phenotypes. This residue is thought to form part of a ''hydrophobic lock'' that stabilizes the closed state of the channel. If this hydrophobic lock is disrupted by the insertion of a charge, the transition energy barrier for MscL gating is destabilized and the channel gates even in the absence of membrane tension. using this observation, researchers have successfully changed the modality of MscL to be sensitive to stimuli such as light and pH simply by chemically modifying the G22 site within the MscL channel. Due to its ability to be triggered by different stimuli and the large pore size, MscL has been proposed as a triggered nanovalve for its use in nanodevices such as a liposome drug delivery system. Here, by utilizing *in vivo,* flux and patch clamp assays, we characterize other neighboring residue that also form part of the hydrophobic lock and we show that the G22 site may not be the best choice for all modifications that change channel modality.

2402-Pos Board B421
Mechanosensor and Gate is Tightly Coupled in the Bacterial Mechanosensitive Channel MscL
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The bacterial mechanosensitive channel MscL is constituted of homopentamer of a subunit with an inner (TM1) and an outer (TM2) transmembrane α-helix. The major issue on MscL is to understand the gating mechanism driven by tension in the membrane. To address this question, we previously performed MD simulations for the MscL opening and found that Phe 78 in TM2 acts as a major tension sensor by its exceptionally strong and stable interaction with surrounding lipids. Neighboring TM1s cross and interact with each other near the cytoplasmic side through hydrophobic interaction between Leu19-Val23 in a TM1 and Gly22 in a neighboring TM1, forming the most constricted hydrophobic part of the pore called gate. Upon membrane stretch, the helices are dragged by lipids at Phe78 and tilted, accompanied by outward sliding of the crossings, leading to a gate expansion. In this study we performed MD simulations of several MscL mutants to get insights into the relationship between the tension sensor F78 and the gate. The GOF mutant G22N shows spontaneous opening without membrane stretch and easier to open compared with WT MscL, while the LOF mutant F78N cannot be opened even under strong membrane tension: these behaviors were qualitatively reproduced by MD simulations with the same conditions used in WT MscL. To test whether the behavior of G22N is independent of the tension sensing at F78, we carried out MD simulations of the double mutant G22N/F78N MscL with and without membrane stretch, and found that G22N/F78N MscL did not show any sign to begin channel opening in the both conditions, suggesting that the tension sensor and the gate of MscL is tightly connected and that the interaction between the tension sensor and lipids is essentially important for the MscL opening.

2403-Pos Board B422

Electrophysiological Characterization of Mechanosensitive Ion Channels in Native Bacterial Membranes

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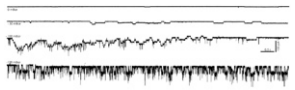
Mechanosensitive (MS) ion channels are membrane proteins that detect and respond to membrane tension in all branches of life. In bacteria, MS channels prevent cells from lysing upon sudden hypoosmotic shock by opening and releasing solutes and water.

Specially prepared giant E. coli spheroplasts of bacteria can be used to study the function of bacterial ion channels in patch clamp experiments. Several native mechanosensitive channels can be measured in E. coli. Although this technique has been used since 1987 it still needs a lot of practice to measure native E.coli membranes.

The spheroplasts don't have a Cytoskeleton and are therefore very fragile. using a planar borosilicate substrate to patch these membranes stabilizes these membrane patches.

The currents shown above are elicited by increasing pressure steps ranging from 0 to - 120 mbar.



A characterization of endogenous mechanosensitive ion channels and overexpressed ones will be shown on the poster.

2404-Pos Board B423

Scanning Ion Conductance Microscopy Allows In-Depth Investigation of Mechanosensitive Ion Channels

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Scanning ion conductance microscopy (SICM) allows measuring simultaneously various structural and functional parameters with nanometre resolution in living cells. We are looking for relevant biological, physiological and medical questions to apply this generally still rarely used technique. There is great interest in mechanosensitive ion channels and their context in pain perception. The function of mechanosensitive ion channels and the pain pathway are still not entirely understood. using SICM, we show that it is possible to investigate and activate mechanosensitive ion channels in P4 cells, a mice hippocampal neuron cell line. Furthermore we are able to demonstrate with this technique a link between the actin network and mechanosensitive channel function. In conclusion, SICM provides a highly informative imaging technique for functional analysis of the mechanisms of mechanosensitive ion channels, which will lead to a better understanding of these channels and thereby to the development of novel therapeutic strategies in treating pain.

2405-Pos Board B424

Models for the Sensitivity of Voltage Gated K Channels to Bilayer Mechanical Stresses

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Ion channel P_{open}values measured in membrane patches typically vary with patch history (seal formation mechanics, stretch, time). Pipette aspiration of cell surfaces causes blebbing, an aspiration-intensity dependent dissociation of bilayer and cortical cytoskeleton. This alters the bilayer's mechanical state, rendering it more symmetrical, disordered, fluidized. Once a gigaohm seal forms, adhesion plus aspiration forces contribute to mechanical stress in the bilayer. For voltage-gated channels (VGCs), including Kv channels, $P_{open}(V,t)$ depends on the bilayer's mechanical state. Aspiration-induced structural changes in the bilayer alter Kv channel P_{open} both irreversibly and reversibly. Because the specifics of how bilayer mechanics affect VGC activity remain unclear, we have amplified the modeling approaches initiated by Tabarean & Morris 2002 (BJ 82:2982) and by Schmidt et al 2012 (PNAS 109:10352) for Shaker and other Kv channels. The Markov models considered are variations on kinetic schemes introduced by Aldrich & colleagues (Zagotta et al 1994 JGP 103:321). In some versions we consider (non-N-type) inactivation and late concerted transitions. We tested model variations for their ability to describe voltage clamp responses of Kv1 Shaker (WT, 5aa, ILT) and Kv3 Shaw without/with pipette aspiration. The main question is how do the features of $I(V,t)$ and $Q(V,t)$ families vary under mechanical stress? We assign mechanosensitivity to one or several of the voltage-dependent transitions and the pore opening/closing transition and show how time courses and $I(V)$ and $Q(V)$ change. A stimulus ''opposite'' to bilayer stretch, in that it increases lipid packing order and bilayer thickness, is hyperbaric pressure. These stimuli, more accurately, fall along a bilayer-mechanical continuum. Since ocean-dwelling organisms from bacteria to cetaceans routinely experience hyperbaric pressures, we extended our analysis to explore, for selected models, how hyperbaric pressures might affect Kv gating. *Funded by NSERC (Canada).

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Novel Structural and Dynamical Features of Water Permeation through an Aquaporin Crystallized at Sub-Angstrom Resolution

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Aquaporins are ubiquitous trans-membrane channels that maintain water homeostasis of the cell by facilitating selective diffusion of water across the membrane while preventing proton diffusion. The selectivity has been suggested to be achieved by two conserved regions located along the pore: the dual asparagine, proline, alanine (NPA) aquaporin signature motif, and the aromatic/arginine (ar/R) selectivity filter. Recently, our collaborators have crystallized a yeast aquaporin at sub-angstrom resolution, the highest resolution achieved to date for a membrane protein. The structure reveals a great deal of novel information on the structure of hydrogen-bonded network of water and protein side chains. In order to complement the experimental results by determining the dynamics and energetics of water diffusion along the channel, we performed molecular dynamics simulations of this impressively high quality crystal structure. The results show disruption of the water chain in both NPA and ar/R regions in this aquaporin, due to characteristic hydrogen-bonding patterns that dictate specific orientations to water molecules. The motion of water molecules is highly correlated on either side of the NPA region. On the other hand, the correlation is reduced at the NPA region, attesting yet another possible mechanism for this region to contribute to a barrier against proton transport. Besides, the NPA region appears as a barrier region with low occupancy for water, a feature not seen in other aquaporins. The correlated motion of adjacent water molecules along with their binary cooccupancies in the ar/R selectivity filter show that water molecules move in pairs in this region. Specific hydrogen-bonding patterns in the ar/R region may also play a role in exclusion of hydronium and/or hydroxide ions. These simulations have helped elucidate the dynamical basis of many intricate features revealed by this new structure.